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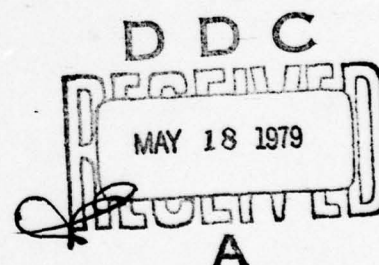
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ENHANCEMENT OF OLFACTORY DISCRIMINATION

Interim Report for 1977-78

March 1979

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Dogs were trained to detect pentyl acetate at a concentration of 10 to the -6.25 of saturated vapor. Three ml of the liquid odorant were then administered orally. Performance in detecting pentyl acetate rose over the next 10-15 days to achieve a stable plateau performance 30-50 percentage points higher than baseline performance. This high performance persisted over the next 58 sessions (days) and following a one week break in testing was reattained after four sessions. Additional studies were conducted that demonstrated that olfactory sensitization of the drop was found to be dependent on the structural similarity of the

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20. sensitizing agent to the compound to be detected. Thus, detection of pentyl acetate was affected by the ingestion of propyl acetate, but not by alpha-ionone.

SUMMARY

Two dogs were trained to detect pentyl acetate at a concentration of $10^{-6.25}$ of saturated vapor. 3 ml of the liquid odorant were then administered orally. In the case of one dog the performance gradually rose over the next 10 sessions to achieve a stable plateau performance 30-50 percentage points higher than the baseline performance. This performance persisted over 58 sessions (of 50 trials/session) following a one week break in testing was reattained after 4 sessions.

The second dog showed a similar increase in performance following ingestion of the odorant reaching a maximum performance in the range of 30-40 percentage points above baseline levels. When the experiment was repeated on the second dog (after performance had returned to baseline) the scores again showed a progressive increase reaching an unstable plateau performance (18 sessions after odorant ingestion) at 10-30 percentage points above baseline. It is concluded that oral administration of a test odorant can markedly enhance performance of dogs in detecting that odorant and that in some cases, at least, this enhancement persists indefinitely. Repetition of the dose does not necessarily lead to further improvements.

A study was made of certain motivational factors potentially capable of influencing performance of dogs working for a water reward on an odor detection task. Performance scores showed no close relation to daily mean water intake during test sessions (rank correlation coefficients were not statistically significant). On the other hand, the highest test scores were obtained when the highest proportion of daily water intake was given as rewards during experimental sessions. Motivation was best sustained by frequent and regularly scheduled sessions uninterrupted by

inactive periods of more than - at most - two days. Individual dogs
may show characteristic patterns of variation in performance.

We have used a two-choice behavioral test apparatus incorporating
two pneumotachometers to measure nasal flow rate and other parameters
in dogs sniffing during an odor detection task. To compare this data
with human sniffing we have asked 5 human subjects to sniff dilute
pentyl acetate through pneumotachometers and report when they detected
the odorant. In marked contrast to dogs in which sniffing consists of
alternate inspirations and expirations, sniffing in human subjects
consists of a series of inspirations superimposed on one longer sustained
inspiration. Parameters for an average human sniff can be taken as
approximately: peak flow rate: 60 l/min; volume: 90 cc; duration 200 m.sec.
The volume is not much greater than the corresponding figure for the dog
but the duration is about twice as long. Mean sniff frequency - 3 sniffs/sec -
was about half the rate of the dog.

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PART I.

ENHANCEMENT OF ODORANT DETECTION

FOLLOWING ODORANT INGESTION.

A. Experiments with dogs.

Introduction

In earlier reports, a new, rather surprising, behavioral assay of olfactory processing was reported. In these experiments, the effect of the oral ingestion of an odorant upon the ability of dogs to detect this odorant or a different odorant in the vapor phase was investigated. In the first study, the odorant ingested was the same as the odorant to be detected. One female dog (4B) ingested 1 ml of α-ionone and was required to detect α-ionone in the vapor phase, and one female dog (2P) ingested 1 ml of pentyl acetate and was required to detect pentyl acetate in the vapor phase. Ingestion of an odorant markedly affected detection of that odorant. Performance initially declined for Dog 2P, increased for a number of days thereafter for both animals, and then returned to baseline performance levels.

In the second study, the specificity of this "sensitization" phenomenon was investigated. The odorant that was ingested was different from the odorant to be detected. One female dog (2P) ingested 1 ml of α-ionone and was required to detect pentyl acetate in the vapor phase, and one female dog (5M) ingested 1 ml of propyl acetate and was required to detect pentyl acetate in the vapor phase. Pentyl acetate is structurally similar to propyl acetate for they are 5 and 3 carbon atom members, repectively, of the homologous series of saturated aliphatic acetates. Pentyl acetate, however, is structurally distinct from α-ionone. If sensitization is a nonspecific phenomenon, then the change in the detection performance for pentyl acetate will not be dependent upon the particular odorant that is ingested. But if sensitization is a specific effect, the change in the detection performance for pentyl acetate will be de-
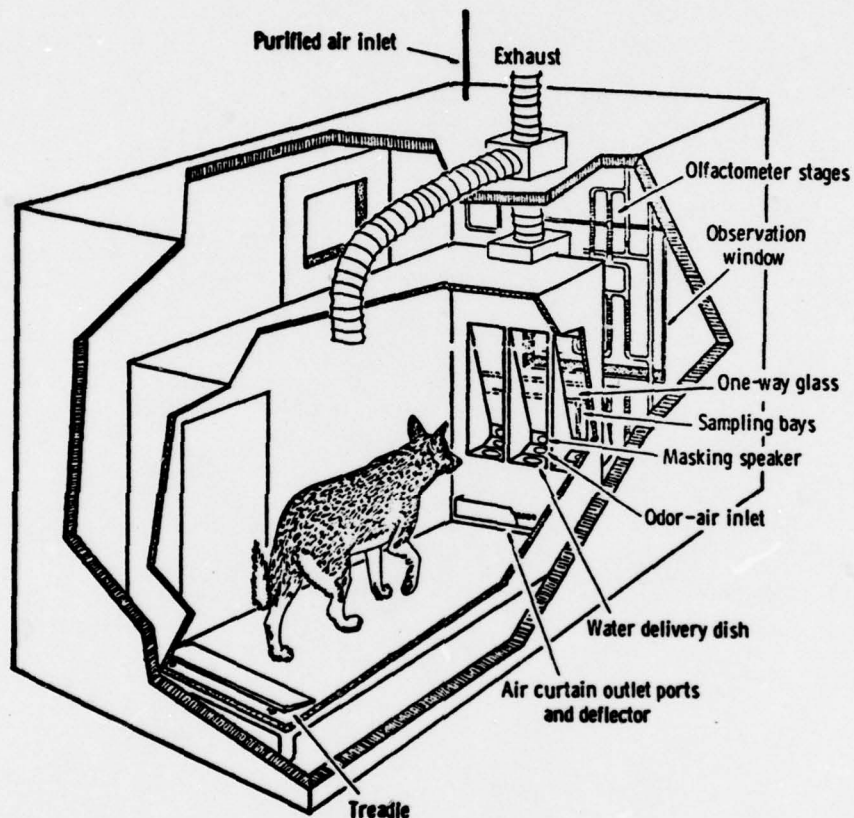
Fig. 1. Simplified view of controlled environment room containing test chamber. The height of the room has been reduced and certain details omitted for the purposes of illustration. (A gas chromatograph and water reservoir bottles normally rest on the roof of the chamber and an air conditioning unit and purification stages are housed on the roof of the room. The vapor saturator is not visible and the olfactometer is shown in simplified semischematic form.) The one-way glass windows normally reflect rather than transmit light from the angle shown here.
(from Moulton & Marshall, 1976)

pendent upon the degree of structural similarity between the ingested odorant and the test odorant. Sensitization was, indeed, found to be dependent upon this structural similarity: Detection of pentyl acetate was affected by the ingestion of propyl acetate, but not by α-ionone.

The present work to be reported examined in greater detail the time course of olfactory sensitization. In particular, we asked the following question: Is the strength and time course of this phenomenon dependent upon the dosage of the ingested odorant?

Method

Subjects and Apparatus

Two German shepherds, several years of age, served as subjects. The chamber consisted of three wind tunnels and a treadle at the rear. Odor or air was directed through each tunnel, with solenoid valves controlling the odor-air sequence to the tunnels. The outputs were connected to the tunnel in such a manner that odor flowed to one and air to the other two. The animals were able to sample the stimuli in stimulus bays cut into the tunnels. Details of the chamber and the olfactometer have been discussed in previous reports and in Moulton and Marshall (1976). Figure 1 provides a simplified view of the controlled environment room containing the testing chamber.

Procedure

Preliminary Training. The olfactory detection performance of a dog was first assessed at a value of vapor saturation of pentyl acetate that resulted in a percentage correct score between 40% and 60%. (For both animals, the concentration was $10^{-6.25}$ of saturated vapor.) By depressing the treadle, the animal initiated a trial. It then had a choice among three streams: two "blanks" and one carrying pentyl acetate. The task for the animal was to detect which stream was

carrying the odorant by breaking, with its head, a photocell beam in the stimulus bay for 5 sec. If the bay chosen was correct, the dog was allowed to drink water for 7 sec from a petri dish in the floor of the bay; during this time the other stimulus bay doors were closed. If the bay chosen was incorrect, the bay door closed forcing the dog's head out of the bay. An intertrial interval of 30 sec then began, during which time stimulus presentation positions were shifted for the next trial. Odor presentation position was determined by a Gellerman sequence of positions.

Training. Following preliminary baseline training, the effect of the oral ingestion of a substance upon the ability of the dogs to detect pentyl acetate in the vapor phase was investigated. Both during preliminary training and training, one or two sessions were conducted daily six days per week for each animal.

Dog 5M. This female dog had not exhibited the sensitization phenomenon upon ingesting a placebo, or 3ml and subsequently 6ml of pentyl acetate. Following a further period of baseline training, the animal ingested 3 ml of pentyl acetate. After about two months of testing, the dog was not tested for 1 week. This was to examine whether sensitization was weakened by "disuse": Would the heightened sensitivity of the animal diminish if the dog did not continue to perform the detection task? If disuse weakens sensitization, then the detection performance of the dog should return to its baseline level.

Dog 4B. This male dog had not exhibited the sensitization phenomenon upon ingesting a placebo or 1 ml of pentyl acetate. Following a further period of baseline training, the dog ingested 3 ml of pentyl acetate. After more than 5 weeks of testing, the performance of the animal was allowed to decrease to chance by having blank air flow

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Dog 5M

Test stimulus: 10$^{-6.25}$ pentyl acetate
Administered: Pentyl acetate

3 ml P

break

Days

Percent correct
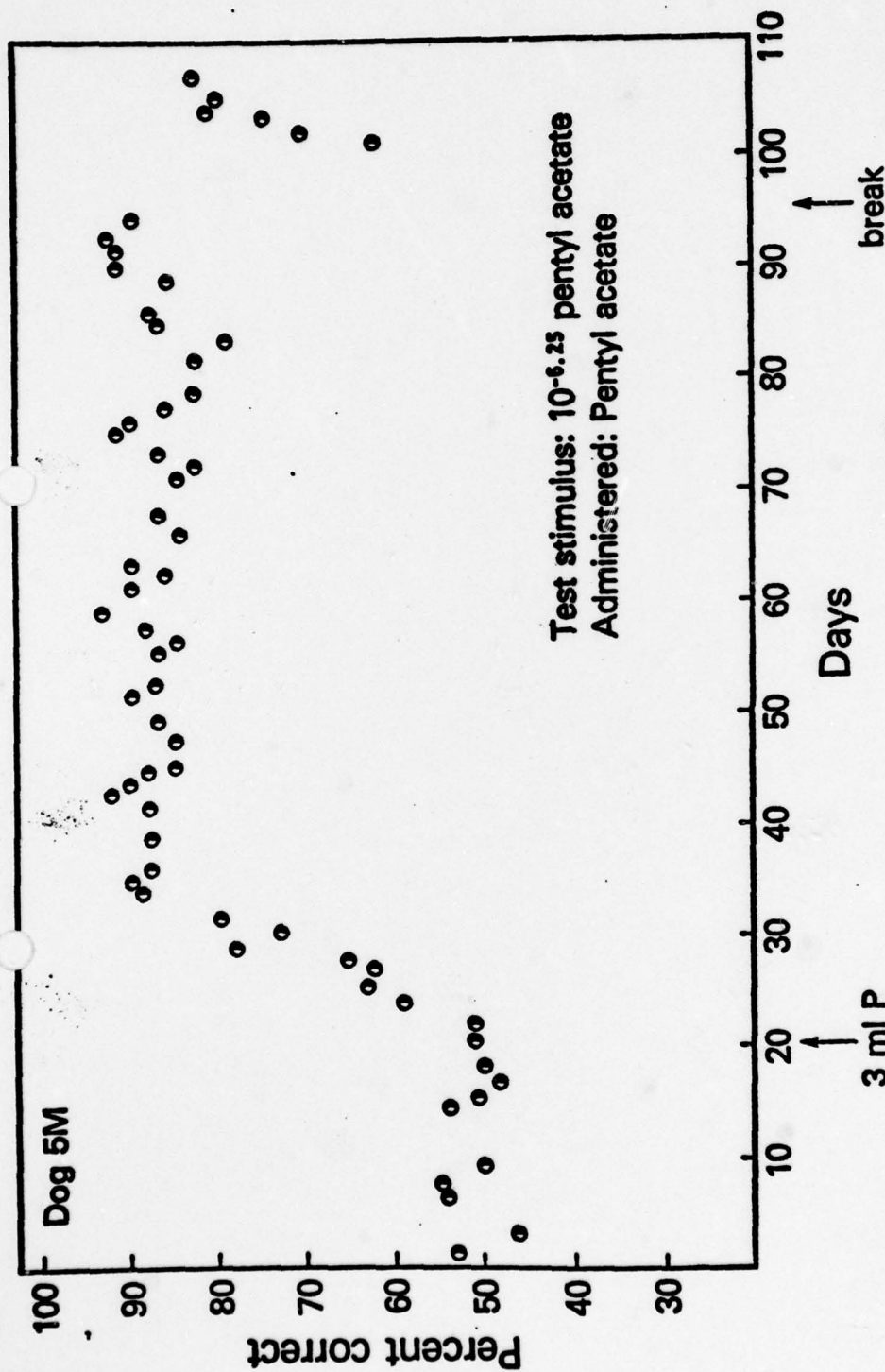
Fig. 2. Dog 5M was trained to detect pentyl acetate diluted to 10$^{-6.25}$ of vapor saturation. Its performance on this odor had stabilized within a range of 45-55% correct (chance score=33%) over for more than 20 days when 3 ml of pentyl acetate in liquid form was administered orally (in a capsule). After about five days performance began to improve to reach a plateau within range of 80-95%. It maintained this level for 45 days at which point the experiment was interrupted for one week to determine if a pause would modify the perform-ance. Upon resumption of testing the performance rapidly ascended to its previous plateau.
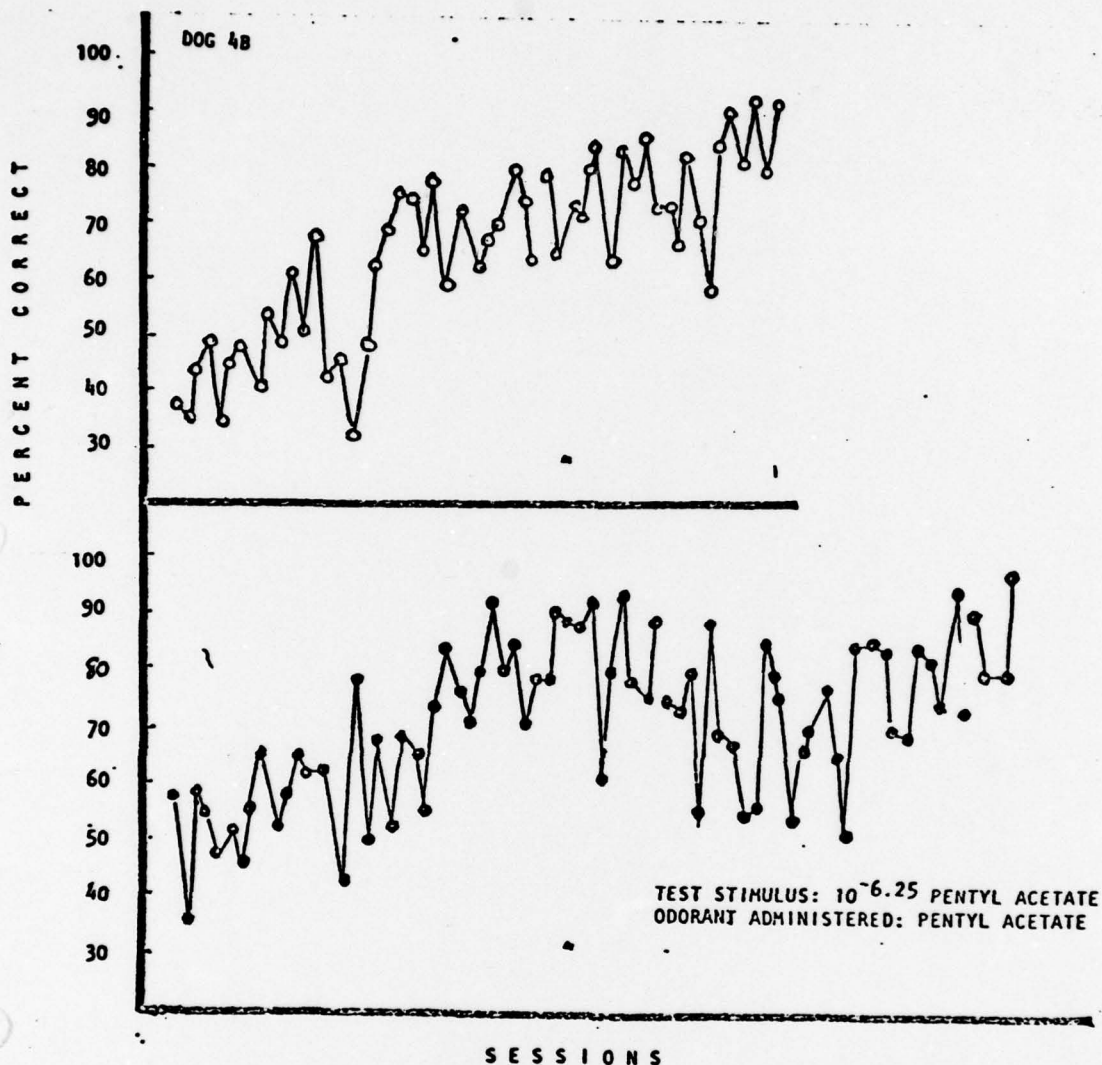
Figures 3a & 3b.   Performance of Dog 4B during baseline pretraining (first 5 sessions in (a) & in (b).) and following ingestion of 3 ml of pentyl acetate. ($10^{-6.25}$ of saturated vapor). The acetate was administered between the 5th and 6th sessions in each test period. (1a)=Test Period I and (b)=Test Period II).

through the odor lines of the olfactometer. Another baseline training period for detecting pentyl acetate then occurred, followed by the dog's ingestion once again of 3 ml of pentyl acetate.

Results

Figures 2 & 3 show the percentage correct response of the dogs as a function of sessions. (Chance performance is 33%.) The last five sessions of baseline training followed by the test period are presented.

Dog 5M. For this animal (Figure 2), the ingestion of 3 ml of pentyl acetate resulted in an increase in performance that did not diminish over a two month period. (On some sessions, the percentage correct score was 100%.) The animal was then not tested for 1 week. When testing was resumed, the initial performance score was at baseline level. But within a few sessions of testing, increments in performance occurred, and the animal reached a detection level that was the same as that achieved before the 1 week stop in testing.

Dog 4B. Figure 3(a) shows the performance of this dog during its first test period. The initial enhancement in performance upon the ingestion of 3 ml of pentyl acetate was followed by an abrupt return to baseline about a week later. Increments in performance occurred thereafter, without a return to baseline after more than 5 weeks of testing. Figure 3(b) shows the performance did occur, but there was great deal of variability from session to session. Nevertheless, after 3 months of testing, detection during the last five sessions was between 70% and 100%.

Discussion

As shown in Figures 2 & 3, the sensitization phenomenon occurred for both dogs following the ingestion of 3 ml of pentyl acetate. But the

time course was _different_ from that discussed in our earlier reports. Upon ingesting 1 ml of an odorant, the detectability of that odorant was enhanced about a week after the ingestion of the particular odorant; this heightened sensitivity eventually diminished over 2 to 3 weeks of testing. Upon ingesting 3 ml of an odorant, the increase in detectability of that odorant did not diminish over several months of testing.

Theoretical Accounts of Sensitization

Two different explanations of this "dual sensitization" effect will now be discussed.

1. It may be possible to give an account of sensitization in terms of the "opponent-process" theory of Solomon and Corbit (1974). The onset and maintenance of a hedonically arousing stimulus is said to create a hedonic a-process and a primary condition called state-A. As a result of the onset of state-A, a hedonic process which opposes A, an opponent-b process is activated. When the stimulus is presented, there is a rise of the A-state to a peak intensity. Shortly afterward, there is a recruitment of the opponent-b process. This b-process, which is slow in its onset and decay relative to the a-process, reduces the intensity of the A-state even though the affective stimulus is still present. When the stimulus is removed, the A-state quickly dies out, but the B-state which is hedonically the opposite of the A-state and is the product of the slowly dying b-process manifests itself. The B-state exists for a period of time and then slowly dies out.

In terms of this opponent-process theory, the ingestion of an odorant may be said to create a hedonic a-process resulting in the initial decrease in the detection performance (A-state) which we reported in our earlier papers. Some time after the onset of the a-process,

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an opponent-b process develops, thereby slowly increasing the detection level. Within a few hours, the ingested stimulus has undergone meta- bolic alteration and a related substance is circulating in the blood stream. With the removal of the ingested odorant, the B-state manifests itself in the increase in the detection performance to a level above baseline. This heightened sensitivity exists for a period of time and then slowly dies out, as revealed by the return to the initial baseline level.

In addition, opponent-process theory predicts that increases in the intensity or duration of the affective stimulus increase the potency of the opponent B-state. As such, as the dosage of pentyl acetate increases, the percentage correct should increase above baseline perform- ance, and the improvement in performance should last for a longer period of time, as was, indeed, shown in Figures 2 and 3.

It should be noted that while the opponent-process theory correctly predicts the temporal dynamics of sensitization, it also predicts the lack of specificity of sensitization. Because opponent-process theory involves central processing, the ingestion of an odorant should result in increased sensitivity to that substance or to a different substance. But our early work (see the Introduction) has revealed the specificity of the "short-term" sensitization effect. Whether the "long- term" sensitization effect (Fig.2 & 3) also has the property of specificity has not yet been determined.

2. A peripheral level account of sensitization is also possible. The ingested odorant partially penetrates into the olfactory epithe- lium and decreases the excitability threshold of the receptors, thereby sensitizing the receptors for a fixed interval of time and increasing

olfactory detectability. Increasing the dosage of the ingested odorant increases the period of heightened sensitivity. (The initial decrement in performance that is sometimes present (see the Introduction) may be an adaptation phase that is distinct from the sensitization phase.) Changes in detection are, therefore, dependent upon the degree of structural similarity of the ingested odorant to the test odorant. As such, this sensitization of specific sites may bear some resemblance to an immuno- logical process (cf. LeMagnen, 1949). Just as the ingestion of an odorant increases the sensitivity for that specific odorant, so, too, does the introduction of an antigen into an appropriate host give rise to the formation of antibodies that will react specifically with that antigen (Rose, Milgrom, & van Oss, 1973).

Future Studies

As it was noted above, the property of olfactory specificity has been studied only for the short-term sensitization effect. We are now investigating the specificity of the long-term effect: Will dogs 5M and 4B show a heightened sensitivity to pentyl acetate upon ingesting 3 ml of ionone? If they do not, an explanation of sensiti- zation in terms of opponent-process theory would be difficult to maintain.

Until now, the short-term and long-term sensitization effects have only been studied in just one dog (5M: see the Introduction and Figure 2). Within the next few months, we intend to see whether both effects can be obtained in other animals as well.

References

LeMagnen, J. Etude d'un phénomène de sensibilisation olfactive.
Comptes Rendus, 1949, 228, 122-124.

Moulton, D.G., & Marshall, D.A. The performance of dogs in detecting
α-ionone in the vapor phase. Journal of Comparative Physiology,
1976, 110, 287-306.

Rose, N.R., Milgrom, F., & van Oss, C.J. (Eds.), Principles of immunology.
New York: Macmillan Publishing Co., 1973.

Solomon, R.L., & Corbit, J.D. An opponent-process theory of motivation:
I. Temporal dynamics of affect. Psychological Review, 1974,
81, 119-145.

B. Experiments with rats.

Introduction

In addition to studies with dogs a series of related studies has been in progress using rats. During the course of these studies it became apparent that an increase in the number of odor discrimination boxes and associated programming and olfactometric systems would greater facilitate this work. This offered an opportunity to redesign these systems to allow their complete automation, to facilitate their production by machinists to reduce possible sources of contamination and to include improved odor presentation and exhaust systems. Three of these boxes have been completed to the point where they are available for interfacing with associated control and odor/air presentation systems. In the case of one box interfacing has been completed and the apparatus is being tested by using it to train 15 rats. We summarize here the design of the apparatus and the results of training. These results already show that this is an effective and rapid method for training rats on an odor detection task.

Method

Although the apparatus and associated methodology are similar to that which we are currently using with dogs they differ in several significant respects, particularly in the use of levers (rather than photocells) to identify correct and incorrect responses. In addition, a three, rather than a two choice design is used.

The apparatus (Fig. 4) presents rats with three wind tunnels, through one of which flows odorized air while air flows through the remainder. Thirsty rats indicate the position of the odor by pressing

a lever to receive a water reward if correct.

The apparatus (Fig. 4) is, in essence, a stainless steel box
(31 x 26 x 22 cms) at the front of which are 3 odor/air presentation
bays. Each bay is a half cylinder of plexiglass, lined internally
with teflon and mounted vertically against the chamber to form a
wind tunnel. Odor or air is delivered to the base of the bay and
exhausted from the top. During training a water cup is inserted into
the rear of each bay in such a way that it faces a small port in the
chamber wall. This port is just sufficient in circumference to allow a
rat to insert its head into it, sample the odor or air flow, and
lick water from the cup. Below each port, and facing into the chamber
is a lever which the rat presses to indicate a choice. After training
is completed, the three water cups are withdrawn and instead a single
water cup is inserted into the left wall of the apparatus.

The apparatus is continuously flushed with filtered air. It enters
from the center of the roof, is dispersed by a porous teflon baffle
and is drawn down through the wire mesh floor. The entire apparatus is
housed in an sound-attentuating chamber. A fan - mounted on the chamber -
draws air from the apparatus and exhausts it. The wall of the apparatus
contains a glass window, and the rat can be viewed through a correspond-
ing window on the chamber wall. The chamber is equipped with a speaker
to broadcast white noise, and a light.

Odor or air is delivered to the bays from an olfactometer. The
positioning of odor to one bay and air to the remaining two bays is
controlled by solenoid valves mounted on the external wall of the chamber.
A random distribution of odor is achieved by sampling (for 40 msec
intervals) the output of the white noise generator, and feeding this
output to a four position stepper. Three of the stepper outputs

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specify the combination of control valves that are activated at each trial.

Rats are first trained to press a lever for a water reward. Thereafter, they train themselves to detect the odor pentyl acetate at a concentration of 10^{-3} of vapor saturation.

Results & Discussion

The performance of rats in this apparatus is shown in Table I. It is clear that there are session-by-session increments in performance until rats reach 90% mean performance level. We have found in previous studies that this is within the plateau performance range attained by fully trained rats detecting this concentration. This method seems to be an effective and rapid one for training rats to perform odor detection tasks.

Rats 1-8

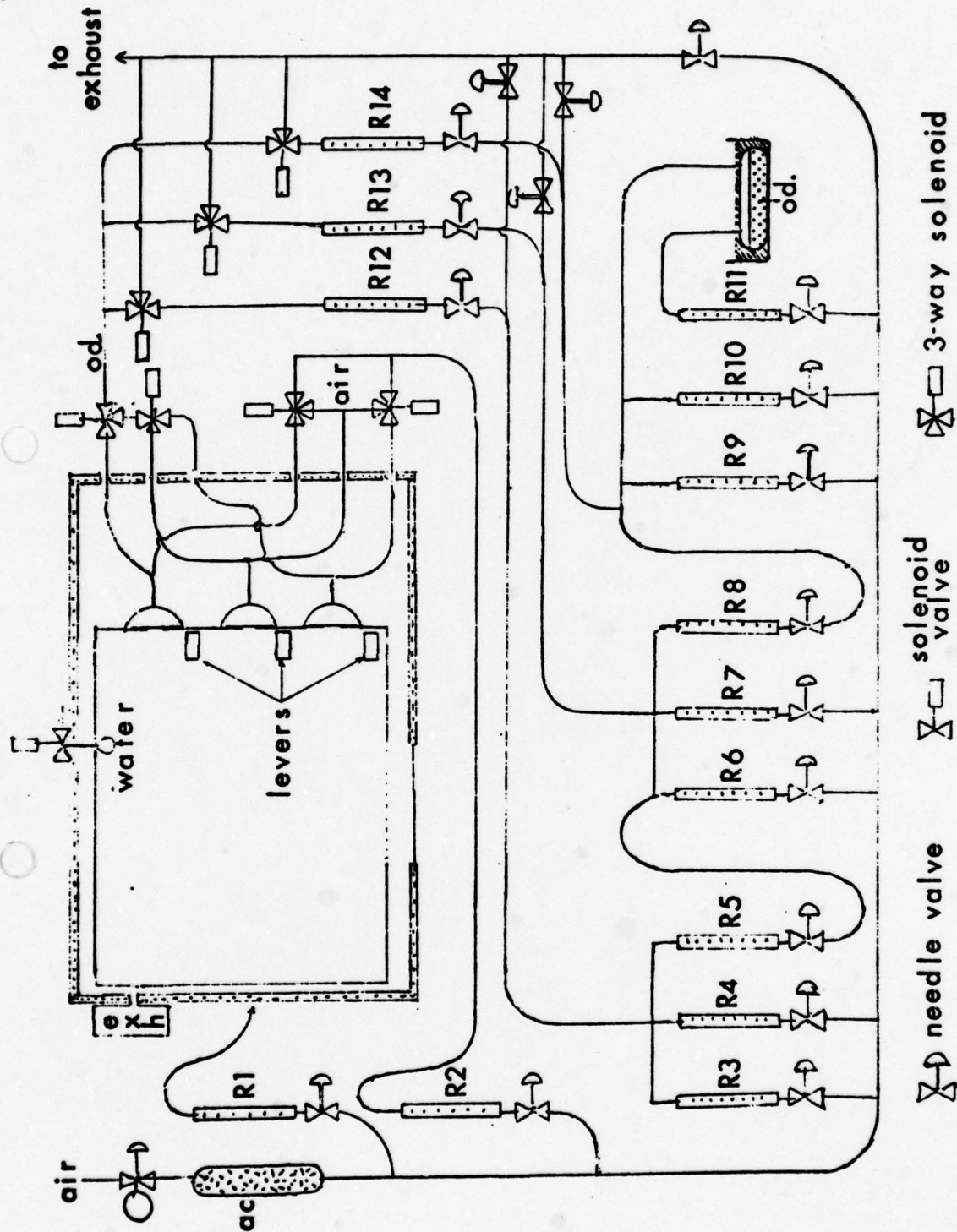| Session (10^{-3})	% Correct								Mean	Range
	1	2	3	4	5	6	7	8		
1	38	29	22	34	42	31	32	37	33.1	22-42
2	56	51	30	31	31	31	31	33	36.8	30-56
3	75	70	40	44	78	67	61	73	63.5	40-78
4	84	87	59	61	87	78	92	91	79.9	59-92
5	93	93	71	62	88	73	93	96	83.6	62-96
6	88	96	84	71	95	87	100	97	89.8	71-100

Table I.

Figure 4.

Fig. 4. <u>Diagram of odorant choice apparatus, olfactometer and valve</u>

<u>switching system</u>.

<u>Behavioral choice apparatus</u>. The apparatus is shown in plan view.

The wall (stippled) of the outer, sound-attenuating chamber supports

the exhaust fan (exh.) as well as a speaker and house light (not shown).

On the side facing the bottom of this diagram is a one-way glass

window. Within the chamber is a stainless steel choice apparatus having

a window that is aligned with that of the chamber. It has three bays

on the front wall for presenting odorized air or pure air. A stainless

steel gate can be lowered to close the port in each bay. When a correct

choice is made, water is delivered to the water cup from a water resevoir.

Pure air entering the roof of the apparatus, is drawn down through the

grid floor and exhausted by a fan.

<u>Olfactometer</u>. An air compressor delivers filtered air through a pressure

regulator to an air purification unit (ac) containing activated charcoal

and silica gel. Thereafter the output of the olfactometer is metered by

14 rotameters (R1-R14). The first two measure the flow of air to the roof

of the odor choice apparatus (R1) and the blank air flow (R2) respectively.

Air flowing through R11 enters the odor saturator (od) comprising the

liquid odorant (od) immersed in a water bath maintained at 22°C. The

odorized air is diluted by air passing through R9 or R10 or both.

After dilution, one fraction is diverted to a stream splitter which has

two outlets. The first flows through R14 while the second is bled to

exhaust. The second main fraction, metered by R8, is diluted by air

flowing through R6 or R7 or both (depending on the degree of dilution

needed) and constitutes the second dilution stage. A fraction is bled off

*The rotameters are of three sizes: ¼" (R1, R3, R6, & R9); 1/8" (R2,
R4, R7, R10, R12, R13 & R14); and 1/16" (R5, R8 & R11), corresponding
to high (¼"), medium (1/8") and low (1/16") flows.

to supply R13 and a bleeder stream, while the remainder goes to the third stage of dilution (R3, R4, and R5) where the process is repeated. This allows dilutions of up to 10^{-6} of vapor saturation to be achieved in successive steps of 10^{-2}. Intermediate flows can also be set, and, if necessary, the system can deliver concentrations down to 10^{-9}. (This, however, is well below the threshold for any compound that we have so far tested with rats).

The three dilution stages we have just described, thus have as their final output three different dilutions of the test odorant. These three dilutions are monitored by R12, R13, and R14 respectively. Each flow can be channeled to a separate box, or, alternatively (as is shown in this diagram) one line can be directed to a single apparatus and the remaining two shunted to exhaust until required. Since the destination of these flows is controlled by solenoids they can be programmed to switch according to a predetermined sequence, thus allowing a series of different concentrations to be tested over a series of sessions.

Switching systems

The odorized air (od.) reaching the apparatus can be delivered to any one of three bays by activating either one or neither of two three-way teflon solenoid valves. Similarly the blank air flow can be switched to any pair of presentation bays by activating either one or neither of two teflon solenoid valves. In this way odorized air is always delivered to one bay and air to the other two.

Although not shown in this diagram, the gas flows to the bays are exhausted by means of lines attached to a source of negative pressure.

PART II.

SOME MOTIVATIONAL FACTORS INFLUENCING THE PERFORMANCE

OF DOGS ON AN ODOR DETECTION TASK

SOME MOTIVATIONAL FACTORS INFLUENCING THE PERFORMANCE
OF DOGS ON AN ODOR DETECTION TASK

In this section we summarize data and observations concerning
motivational factors controlling performance of dogs on an odor de-
tection task with particular reference to the role of water deprivation.

Introduction

Behavioral conditioning techniques are widely used to study sensory
capacities in animals. Operant methods, in particular, are well suited
not only for laboratory experimental studies but also for training animals
to seek out and respond consistently to specific sensory cues - a dis-
tinctive odor, for example. The testing procedures that we have described
in previous reports combine both uses for the purpose of establishing
reliable measurements and maximizing performance; throughout a given testing
series dogs continue to receive training - the progress of which is
weighed against our performance criterion - for each successively lower
test stimulus concentration. The usefulness of this procedure is neither
restricted to laboratory testing nor to olfactory measurement; the same
approach can be profitably applied in training dogs to perform specific
odor detection tasks in the field.

A major variable affecting success in either setting, however, is
motivation of the animal to perform. Following a period of food depri-
vation for example, small quantities of food can be delivered in a way
first to isolate and selectively reinforce particular "segments" of
behavior: these segments are thus linked together as a sequence consti-
tuting the desired performance. By appropriate manipulation of depri-
vation times and reward quantity, motivation to perform can be maximized.
It is upon this process of determining best parameters - particularly

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for reward other than food that success ultimately depends. Confidence
that an animal is closely attending to and extracting a maximum of
stimulus information is thus largely a matter of knowing that detection
will occur within definable limits of variability, as a function of
stimulus quantities known to be present.

In studies reported here and in previous reports, however, we
have used water as a reward in olfactory work. Water reward avoids
the potential problems of stimulus and adaptation due to food odors
in the testing environment and especially the close proximity and direct
access, these odors may have had to the olfactory receptors. The
question is: does water deprivation and the use of water in small quanti-
ties as a reward produce consistently high levels of performance motiva-
tion?

Method

Average amounts of water delivered daily to 5 german shepherds,
weighing 19.3 -22.7 Kg, were recorded over a period of 23 months. In
addition to this amount, the dogs also obtained small quantities from
moistened dog food (present both as added water and derived metabolically).
However, this component remained relatively constant. A further potential
source of water is surface water left on runs following the daily washing
of these areas. Since great emphasis was placed on drying these surfaces
before dogs regained access to them it is unlikely that any significant
quantity, if any, was obtained from this source.

Recorded water intake was of two kinds: that obtained by the dogs
as rewards in the test apparatus and additional amounts given to the dogs
following completion of their daily sessions during hot weather and at
times of low relative humidity. This was necessary to maintain animals
in good health and with adequate food intake.

Performance motivation was measured by the number of trials completed and by the consistency of session-to-session scores.

Results

The 23 month daily average for all dogs by months is 312 ± 137 ml (mean ± standard deviation). Calculated across individuals the value is 312 ± 36 ml. The larger variation over calendar months partly reflects seasonal differences in temperature and relative humidity which, although absent in the test chamber and attenuated (due to temperature control) in the indoor runs, are not eliminated. During phases of detection testing when higher odor concentrations were presented (and thus resulted in higher percentage of rewarded trials) average water intake quantities remained generally high independent of time of year. Conversely, with lower concentrations there were fewer rewarded trials and at those times daily water intake decreased.

Performance motivation showed no close relation to daily mean water intake during test sessions. Performance was maintained provided that the dogs did not receive more than about 800 ml day (averaged over a long period). If these condition were met the total number of experimental trials executed per session - or per day, if more than one session - appears to correlate best when motivation was sustained by another factor: frequently and regularly scheduled sessions, uninterrupted by inactive periods of more than - at most - two days.

A further study was made of the relation between mean water intake and performance motivation. In this case, performance motivation was measured by the number of trials each dog would perform per session. Data were compared for 12 week testing periods covering concentrations

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of $10^{-3.5}$ - 10^{-5} and 10^{-5} to $10^{-6.5}$. For each period, daily water intake averages (by weeks) were divided at the 23 month mean of 312 ml into two groups (i.e., 312 ml vs 312 ml intake). Correlations between water intake and trials/session were then obtained for each of the four subgroups. Rank order correlation coefficients (r_s) were as follows:

$$
\begin{array}{lcc}
 & \bar{X} & \bar{X} \\
10^{-3.5} - 10^{-5} & -0.26 & 0.03 \\
10^{-5} - 10^{-6.5} & 0.03 & -0.20
\end{array}
$$

None of these values approaches statistical significance. This tends to confirm the conclusion that independent of task difficulty, average amounts of water obtained throughout testing may vary widely with no obvious effect on the average number of trials executed. But note that it is common to find large day-to-day variations in the number of trials that animals will perform and thus in amount of water they obtain for a given percentage of correct trials. To some extent, patterns of variation are characteristic of particular dogs. For example, a dog may show a total of 80 or more trials/day over two days typically followed by a session in which it performs no more than 35 trials with percentage correct scores remianing high and very nearly the same. Another dog may frequently alternate between larger and smaller numbers of trials performed.

These and other experiences have yielded some further conclusions concerning the use of water as a reward. During initial training with new dogs, 24 hr. water deprivation schedules appear to produce a much greater need for water than occurs in experienced animals. We typically have begun with supplementing amounts given in the laboratory with measured quantities given in the run. As training progresses supplemental

water must be reduced to sustain performance. By increasing the number of trials, many of which will, initially, be rewarded, dogs learn to obtain increasing percentages of water in the test apparatus. Holding daily water intake totals constant - divided between that obtained in the laboratory and given in the run, has not proved useful in this respect. What seems to happen with an increase in testing experience is a reduction of resetting of water needs to lower levels. As the detection task becomes more difficult and fewer rewards are delivered, it becomes increasingly important that dogs be given miximum opportunity to obtain water in the test apparatus. Supplementary daily intake at those times by water in the test apparatus has generally proved to be disruptive of performance. If, in fact, the need to obtain water can, as this suggest, be situationally modified and can vary by adaptive mechanisms over a broad range as we have observed it is not suprising that motivational levels vary, inpart, independently of water needs. Regular opportunities to work for water in the laboratory seem to serve as a strong secondary reinforcer.

Conclusions

This analysis shows that water reward can achieve consistently high levels of performance motivation particularly under the following conditions (1) As large as possible a percentage of daily water intake (or the total) should be given as rewards during experimental sessions, this generally requires that reward quantity be set as large as possible, short of that producing satiation, prior to the desired number of trials. Reward quantities of 6-7 ml for between 50-100 trials (assuming a long term average of about 70% rewarded trials) have proved adequate. (2) Water content of food should remain constant as should intervals between feeding and experimental sessions. (3) Sessions should be conducted on a very regular one or two/day basis.

PART III.

QUANTITATIVE ANALYSIS OF NASAL

AIR FLOW DURING SNIFFING.

Introduction

In experiments described in previous reports we have established that when a dog investigates an odor with its nose it sets up bouts of sniffing which can be defined by up to seven descriptors (sniff volume, fow rate, amplitude, frequency, etc.). The complexity of these patterns varies according to the concentration or type of odorants and with the individual dog. For example, at one extreme, one dog detecting high concentrations of pentyl acetate, sometimes showed only one, high volume, high amplitude sniff associated with the correct identification. At the other extreme, urine elicited a sustained sniffing bout, characterized by low amplitude sniffs and a complex internal structure. These measures are made with two pneumotachometers each behind a sniffing port which, in turn, are the core components of a two choice behavioral test apparatus.

Such curious results raise the question: how does this compare with human sniffing? We searched the literature but found little relevant to this question. Apparently no quantitative attempts have been made to characterize human sniffing patterns when the subject was attempting to detect an odorant. On the other hand, penumotachograms have been published for nasal breathing under different conditions. These show an alternating pattern of inspiration and expiration with a lower peak in inspiration than in expiration (probably due to the collapse of the nostrils at high flows during inspiration). During rapid nasal breathing (about 1 inspiration/sec) the maximum inspiratory flow rate is about 40 l/min. During normal quiet inspiration it is about 30 l/min. No reference is made to individual variations.

We therefore used a pneumotachometer and associated equipment to derive measures of sniffing flow rate and duration in human subjects.

Methods

The mode of operation of the pneumotachometer and the associated transduction, amplification and recording systems have been described in previous reports. In this experiment we attached a cone to the front of the pneumotachometer into which the subject inserted his or her nose. The odorant was contained in a cuvette resting in the floor of a tube attached to the opposite end of the pneumotachometer (as in experiments with dogs). The subject was instructed to hold the pneumotachometer and to sniff into it until he or she detected an odor. The odorant tested was a solution of pentyl acetate diluted to 0.1% by volume (as described in previous reports). Five subjects were tested: two females and three males.

Results and Discussion (Fig. 5)

Subjects showed such individual variability in sniffing patterns as to defy all but a few generalizations. In all cases, sniffing was primarily a sustained inspiration on which sniffs were superimposed. This is in marked contrast to dogs in which sniffing is a series of alternating inspirations and expirations. All but one woman showed a pattern of short sniffs with mean durations in the range of 171-293 m.sec. and mean volumes in the range of 42-237 cc. (The exceptional woman sniffed in prolonged inspirations with a mean duration of 547 m.sec. and a mean volume of 398 cc). Normal sniffs tended to fall into those with a shorter mean duration and those with a longer mean duration. Sniffs with shorter mean durations ranged from 171-213 m.sec. and had mean

volumes of 42-92 cc.   Those with longer mean durations ranged from 230-293 m.sec. and had mean volumes of 99-237 cc.

The volume of odorous air sniffed before the odorant was detected was 1668 cc (4 sniffs) in the case of one female subject.   Generally 3-4 sniffs were required before detection occurred.

As a first approximation the average flow rate for a human sniff is about 60 l/min, volume is about 90 cc with a duration of 200 m.sec. The volume is not much higher than that of the dog but the duration is about twice as long.

When subjects attempted to sniff rapidly the fastest rate attained was 7½ sniffs/sec.   The mean frequency was 3 sniffs/sec.

In Fig. 6 a typical sniffing bout for a dog is shown for comparison.
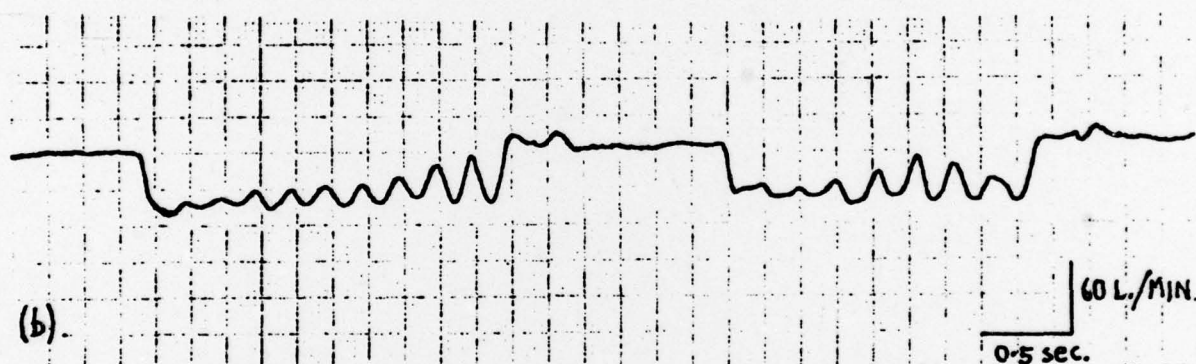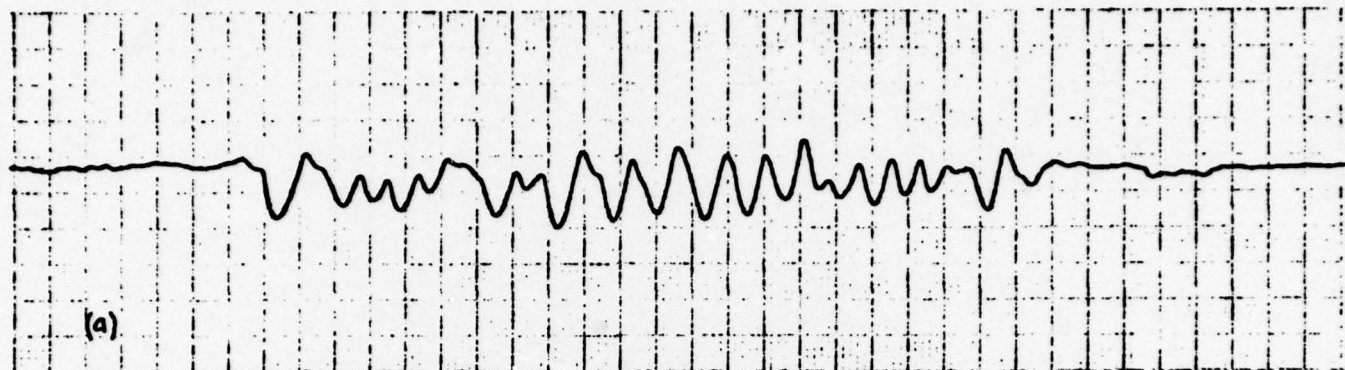
Fig. 5. Pneumotachographs for 2 human subjects sniffing dilute pentyl acetate (a) and a blank (the diluent: ethylene glycol). (b)
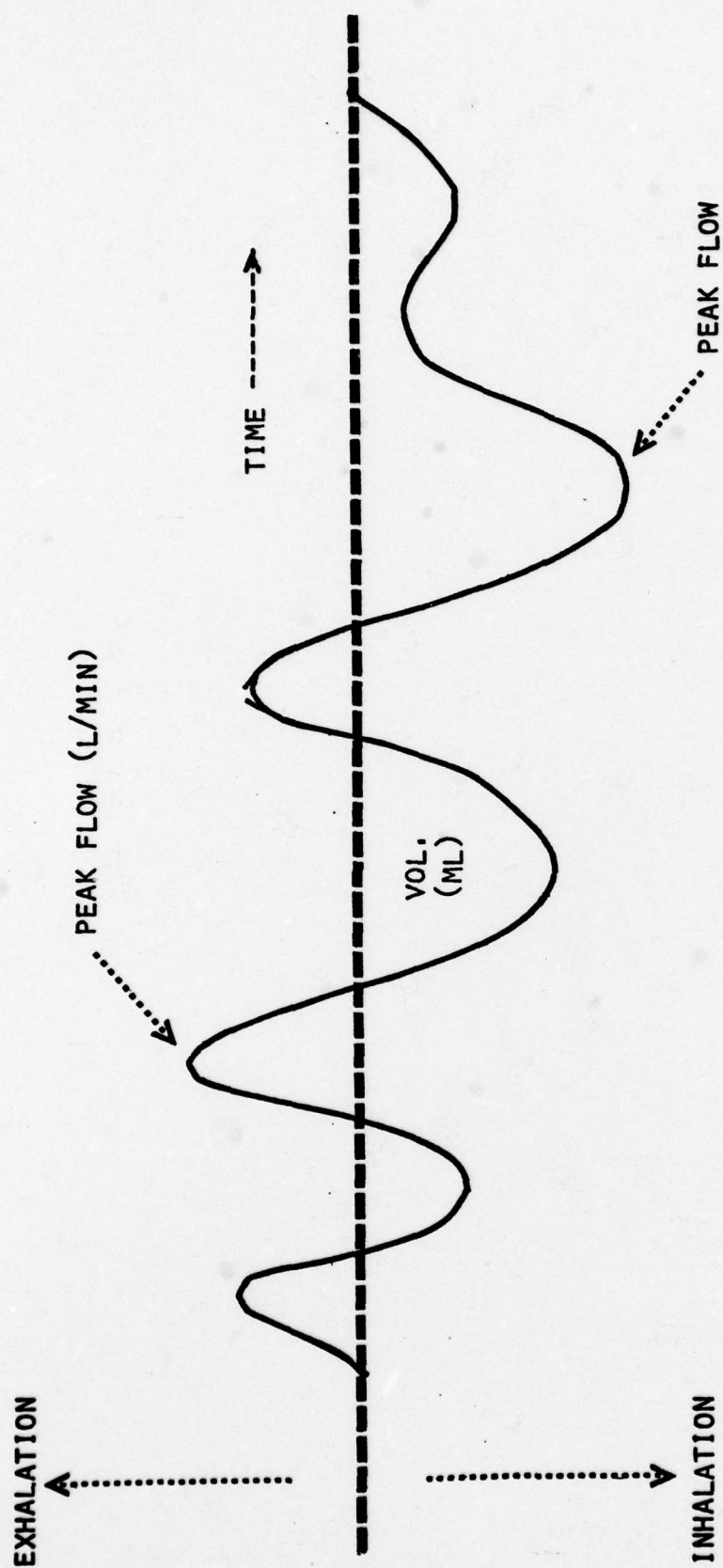
Fig. 6 Representation of a sniffing bout as it appears in penwriter records from which data are quantified. Three exhalations and three inhalations (sniffs) are shown. The area beneath each segment of the curve (from baseline to baseline) gives a measure of volume. The average length of sniffing bouts of the type shown is around 1 - 1.5 sec.

PRESENTATIONS RESULTING FROM WORK SUPPORTED BY THIS GRANT

1) D.G. Moulton:

    Quantitative analysis of olfactory performance in dogs.
    2nd International Symposium on Canine Olfaction. Pennsylvania
    State University, State College, Pennsylvania. 20th & 21st
    May, 1978.

2) D.G. Moulton, D.A. Marshall and M.Z. Deluty. Enhancement of
    olfactory response to odorants following their administration
    to rats and dogs. Third Congress of European Chemosensory
    Research Organization. University of Pavia, Italy. 10-14th
    September, 1978.